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Synthesis and evaluation of novel 8,6-fused bicyclic peptidomimetic compounds as interleukin-1β converting enzyme inhibitors

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Abstract—Two novel 8,6-fused bicyclic peptidomimetic ring systems were synthesized utilizing olefin metathesis as the key reaction for the formation of the eight-membered ring. Both peptidomimetic scaffolds were further elaborated into potent ICE inhibitors, with numerous compounds exhibiting caspase-1 IC_{50}s less than 10 nM.

Cytokines are important signaling molecules that are essential to immune and inflammatory responses in mammals. Much attention in recent years has been given to interleukin-1β (IL-1β), a cytokine known to play an important role in the pathophysiology of articular joint destruction.[1,2] Modulating the levels of IL-1β is therefore believed to be a promising strategy for the treatment of inflammatory diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). IL-1β converting enzyme (ICE), also known as caspase-1, is known to be involved in the processing of biologically inactive proIL-1β to the mature cytokine.[3,4] Therefore, direct inhibition of ICE should provide a means of controlling IL-1β levels therapeutically.

ICE is one member of a family of at least 12 human enzymes referred to as 'caspases.' The term caspase was proposed to describe cysteine proteases sharing sequence homology and a preference for an aspartate residue at the P1 position of their substrates.[5,6] Since the report of the potent reversible tetrapeptide ICE inhibitor Ac-YVAD-CHO (**1**),[7] numerous other reversible and irreversible peptidomimetic inhibitors have been reported.[8] Pralnacasan® (**2**), a selective reversible ICE inhibitor which progressed into late stage clinical trials for RA before being withdrawn for toxicological issues, is perhaps the most studied and successful inhibitor designed to date.[9] Pralnacasan® possesses a bicyclic core which effectively constrains the P2–P3 region of the inhibitor in a preferred conformation and utilizes a prodrug to mask the acidic character of the required aspartic acid recognition element, making the compound orally bioavailable. The lactone acetal prodrug of **2** is reported to undergo enzymatic hydrolysis under physiological conditions to provide the bioactive form of the drug (**3**) (see Fig. 1).

We elected to follow a related approach for the synthesis of ICE inhibitors, constraining the P2–P3 region in the form of an 8,6-fused bicyclic ring system. We believed that bicyclic peptidomimetic compounds such as **4** would possess all the necessary features to function as ICE inhibitors, providing the desired conformational constraint for properly orienting the P1 aspartate residue and the P4 residue believed to impart selectivity between caspase isoforms.[9,6] Inhibitors such as **4** should be readily accessible from versatile bicyclic cores such as **5** (Scheme 1). We envisioned **5** coming from an eight-membered ring closure on **6** via olefin metathesis.[10] Compounds **5a** and **b** (Scheme 2) are ideally functionalized for elaboration into potential inhibitors. Both

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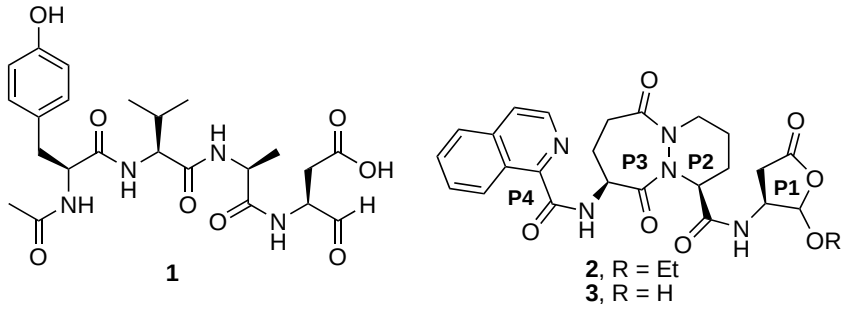
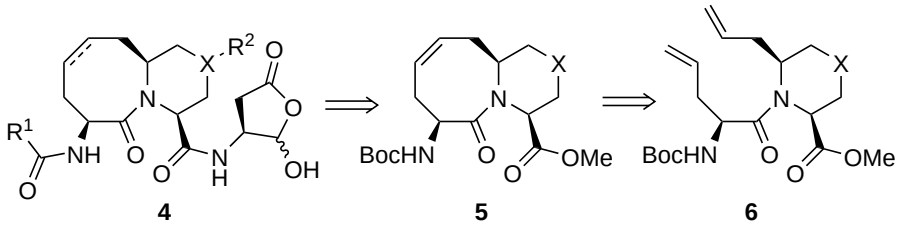
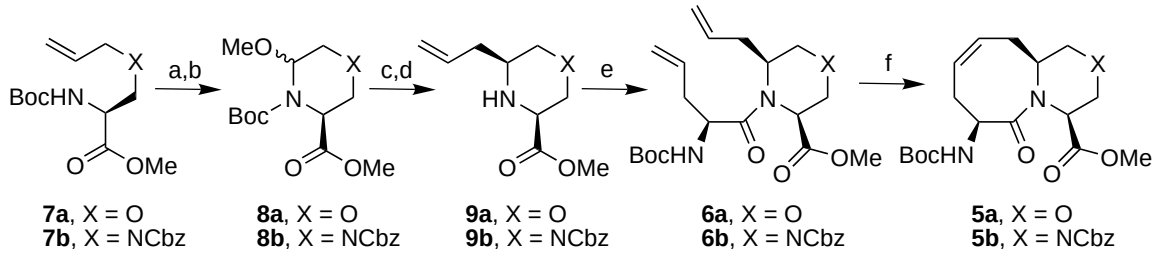


Figure 1. Known ICE inhibitors.



Scheme 1. Retrosynthesis.

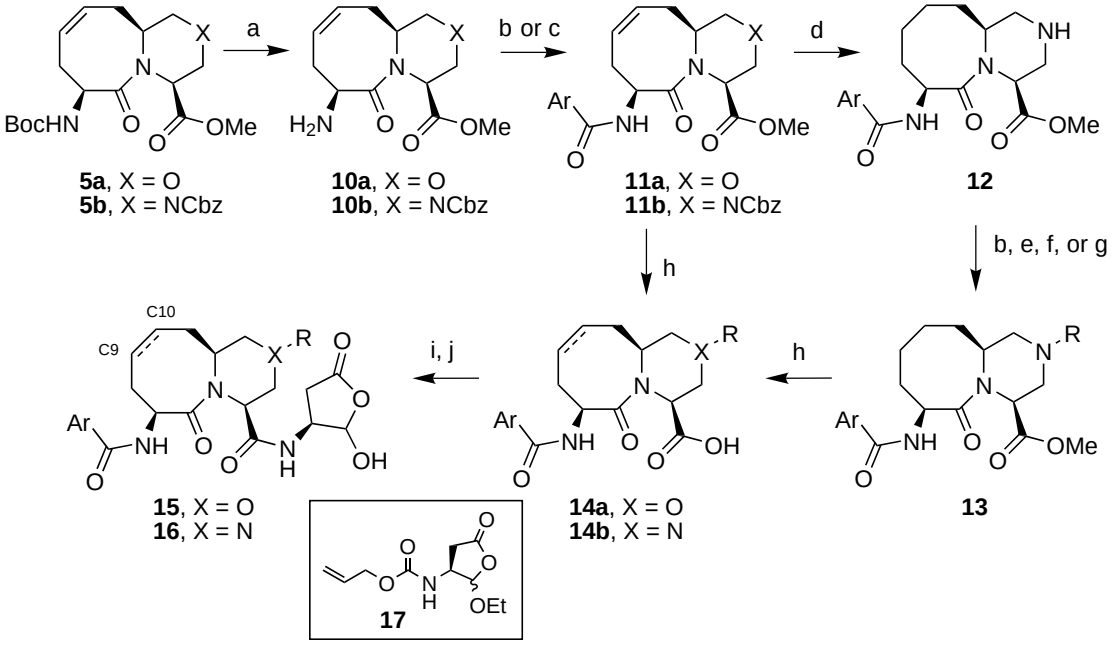


Scheme 2. Synthesis of **5a**,**b**. Reagents and conditions: (a) O_3, 10:1 CH_2Cl_2/MeOH, −78 °C; then DMS; (b) TsOH, MeOH; (c) allyl trimethylsilane, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C; (d) $SOCl_2$, MeOH or TFA, CH_2Cl_2; (e) *N*-Boc-allylglycine, IIDQ, THF; (f) first generation Grubbs catalyst, CH_2Cl_2.

dipeptide scaffolds possess an ester terminus that can be easily hydrolyzed, providing a handle for attachment of the required aspartic acid aldehyde recognition element. Additionally, the nitrogen terminus can be deprotected and derivatized to explore the P4 region of our inhibitors. Furthermore, **5b**, where X = NCbz, possesses one additional site of diversification to explore further binding in the S2 pocket of the enzyme.

The synthesis of the bicyclic cores **5a** and **b** commenced from readily available allylated amino acids **7a**[11] and **7b**,[12] respectively (Scheme 2). Ozonolysis of **7a**,**b** in 10:1 CH_2Cl_2/MeOH followed by reduction of the ozonide with dimethylsulfide yielded a crude mixture of the hemiaminal and **8a**,**b**. The aldehyde was never observed, due to rapid cyclization of the Boc protected nitrogen onto the newly formed aldehyde. To drive the product mixture to the desired methyl aminal **8a,b**, the crude reaction solution was concentrated and treated with TsOH in MeOH, yielding crude **8a**,**b** in 50–78% yield. Although methyl aminals **8a**,**b** were isolable, they were rather labile and were typically used immediately without purification. Allylation of **8a,b** with $BF_3{\cdot}OEt_2$ and allyltrimethylsilane in CH_2Cl_2 at −78 °C, and subsequent treatment of the crude product with TFA/CH_2Cl_2 or $SOCl_2$/MeOH yielded a single stereoisomer (**9a**,**b**) in each case in good yield (60–70%).[13] The coupling of morpholine **9a** and piperazine **9b** with *N*-Boc-allylglycine was originally problematic under standard carbodiimide coupling conditions. We were eventually able to obtain acceptable yields of **6a**,**b** (40–60%) in this coupling by employing isobutyl 1,2-dihydro-2-isobutoxy-1-quinoline carboxylate (IIDQ) with excess *N*-Boc-allylglycine (3-5 equiv.) in very concentrated THF solutions.[14] Completion of the bicyclic scaffolds (**5a**,**b**) was accomplished via olefin metathesis. Typical metathesis conditions involved refluxing **6a**,**b** in CH_2Cl_2 (0.5–0.75 M) in the presence of first generation Grubbs catalyst (15–30 mol%) and delivered the desired bicyclic core (**5a,b**)[15,16] in 50–60% yield after purification.

After removal of the Boc protecting group upon treatment of **5a**,**b** with TFA in CH_2Cl_2, the crude amines were converted to a variety of aryl amides through either carbodiimide mediated couplings with the required carboxylic acid or by reaction of the crude amine with commercially available acid chlorides (Scheme 3). The isolated yields for this two step sequence were generally between 80% and 95%. Once the P4 aryl amide was introduced, the piperazine nitrogen of **11b** was deprotec-



Scheme 3. ICE inhibitor synthesis. Reagents: (a) TFA, CH_2Cl_2; (b) acid chloride, Et_3N, CH_2Cl_2; (c) $ArCO_2H$, HOBt, EDCI, CH_2Cl_2; (d) H_2, Pd/C, MeOH; (e) sulfonyl chloride, Et_3N, THF; (f) alkyl halide, Et_3N, THF; (g) organo-isocyanate, THF; (h) LiOH, THF/H_2O; (i) **17**, 1,3-dimethylbarbituric acid, $Pd(Ph_3P)_4$, then HOBt and EDCI; (j) TFA, CH_3CN/H_2O.

ted with accompanying olefin reduction upon treatment with H_2 and 10% Pd/C in MeOH. The crude amine (**12**) was isolated upon filtration and concentration of the reaction mixture, but was used without purification. A small sampling of substitutions for the piperazine nitrogen was selected and crude amine **12** was elaborated by standard alkylation, acylation, or sulfonylation procedures leading to **13** in generally good yield. The methyl esters of intermediates **11a** and **13** were hydrolyzed with LiOH in 3:1 THF/H_2O to yield carboxylic acids **14a**,**b** in near quantitative yield.

The aspartic acid aldehyde residue was introduced utilizing intermediate **17**. The free amine of compound **17**[17] was prepared in situ upon treatment of **17** with dimethylbarbituric acid and $Pd(Ph_3P)_4$ in CH_2Cl_2 at room temperature. Once deprotection of the amine was complete (typically <15 min), acids **14a,b** were added as solutions in either DMF or CH_2Cl_2, followed by the addition of HOBt and EDCI. The coupling was generally complete within 2 h and the isolated crude product was treated with TFA in CH_3CN/H_2O to effect hydrolysis of the ethyl acetal to obtain final compounds **15**[18] and **16**.[19]

It is well known in the area of ICE inhibitor synthesis that caspase-1 has a strong affinity for large hydrophobic substituents in the P4 pocket, as well as the requirement for an aspartic acid residue at P1 previously described. To evaluate the ability of our bicyclic dipeptide scaffolds to function as ICE inhibitor scaffolds, we synthesized a small set of aromatic amides at P4. We had previously established a preference for meta-substituted benzamides and fused bicyclic aromatic amides at P4[20] and these trends were again observed for the morpholine-containing ring system (**15**). Among the ten compounds synthesized with P4 variations (**15a**–**j**), five exhibited caspase-1 IC_{50}s < 10 nM.[21] These better performing compounds were also screened in a THP-1 whole cell assay measuring IL-1β production.[22] All five compounds performed well in the whole cell assay, with two exhibiting THP-1 IC_{50}s < 100 nM. To explore the effect of potential conformational changes invoked by the unsaturation in the 8-membered ring on activity, we hydrogenated compounds **15h**–**j** to obtain **15k**–**m**. Direct comparison between these analogs and their unsaturated counterparts showed only a slight improvement in potency, perhaps indicating minimal conformational changes were induced by saturation of the 8-membered ring. This result was contrary to that observed for a related 8,5-fused bicyclic scaffold.[20]

Next we decided to evaluate the effect of having a piperazine as the 6-membered ring of the bicyclic core. Compounds **16a**–**h** were synthesized and screened as described previously. For our initial investigations on the piperazine containing ring system (**16**), we elected to hold the P4 substituent constant as a simple benzamide. All eight of these compounds were found to be quite potent, particularly in the caspase-1 enzyme assay (IC_{50}s < 10 nM), but significant fluctuations in activity were observed in the THP-1 assay. Amides **16c** and **d**, urea **16g**, and tertiary amine **16b** all exhibited a 5–7 times loss in potency when compared to unsubstituted **16a**. This result is probably not due to the inability of the enzyme to accommodate substitution at this position, because several substituents actually resulted in slight increases in activity. Two different sulfonamides (**16e** and **f**) and a benzyl carbamate (**16h**) were well tolerated. These fluctuations in the whole cell activity are more likely related to changes in the cell permeability of the inhibitors. We attempted to further optimize scaffold **16** by incorporating a more potent P4 substituent in

Table 1. Biological activity for compounds **15** and **16**

Compound	Ar	R^1	C9–C10 olefin	THP-1 (nM)	Casp-1 (nM)	Casp-3 (nM)	Casp-8 (nM)
15a	Ph	—	Yes	—	130	$>10^4$	7900
15b	2-ClPh	—	Yes	—	33	$>10^4$	448
15c	3-ClPh	—	Yes	118	7	$>10^4$	1550
15d	4-ClPh	—	Yes	—	74	$>10^4$	3770
15e	2-CF_3Ph	—	Yes	—	50	$>10^4$	670
15f	3-CF_3Ph	—	Yes	132	9	$>10^4$	4190
15g	4-CF_3Ph	—	Yes	—	135	$>10^4$	6450
15h	2-Benzothiophenyl	—	Yes	57	3	$>10^4$	1600
15i	2-Naphthyl	—	Yes	62	3	$>10^4$	2590
15j	1-Isoquinolinyl	—	Yes	182	3	$>10^4$	1585
15k	2-Benzothiophenyl	—	No	178	1	$>10^4$	429
15l	2-Naphthyl	—	No	60	1	$>10^4$	911
15m	1-Isoquinolinyl	—	No	101	1	$>10^4$	264
16a	Ph	H	No	245	10	$>10^4$	384
16b	Ph	Me	No	1820	5	$>10^4$	247
16c	Ph	Ac	No	1260	6	$>10^4$	481
16d	Ph	COPh	No	1420	7	8070	38
16e	Ph	SO_2Me	No	174	6	$>10^4$	384
16f	Ph	SO_2Ph	No	159	2	$>10^4$	106
16g	Ph	CONHPh	No	1690	5	$>10^4$	601
16h	Ph	Cbz	Yes	125	1	3260	13
16i	2-Naphthyl	H	No	75	1	$>10^4$	506
16j	2-Naphthyl	SO_2Me	No	46	1	$>10^4$	283
16k	2-Naphthyl	SO_2Ph	No	153	1	$>10^4$	50
16l	2-Naphthyl	Cbz	Yes	106	1	4850	5
16m	1-Isoquinolinyl	H	No	156	1	$>10^4$	595
16n	1-Isoquinolinyl	SO_2Me	No	168	1	$>10^4$	917
16o	1-Isoquinolinyl	SO_2Ph	No	87	1	$>10^4$	160
16p	1-Isoquinolinyl	Cbz	Yes	154	1	$>10^4$	4

conjunction with the better performing piperazine P2 substituents (**16i**–**p**). For this investigation, we selected the 2-naphthyl and 1-isoquinolinyl groups as our preferred P4 substituents, and hydrogen, methylsulfonamide, phenylsulfonamide, and Cbz as our preferred piperazine substitutions. These efforts resulted in very potent enzyme inhibitors, with all 8 analogs possessing IC_{50}s less than or equal to 1 nM. Unfortunately, only modest improvements were observed in the THP-1 whole cell assay.

To establish that our compounds were selectively inhibiting caspase-1 over other caspases, we screened our compounds against caspase-3 and caspase-8. Though many of our compounds were low nanomolars inhibitors of caspase-1, nearly all had little or no activity at caspase-3 and most of them were fairly selective against caspase-8 (>100×). It is worth noting though that the selectivity over caspase-8 did fluctuate significantly with certain structural changes. The morpholine-containing inhibitors **15** were more selective for caspase-1 over caspase-8 than the piperazine-containing inhibitors **16**. Furthermore, several R substituents (R = COPh and Cbz) in **16** were found to erode selectivity significantly, yielding potent (<40 nM) caspase-8 inhibitors (**16d**,**h**,**l**,**p**) (see Table 1).

In summary, we have synthesized two novel 8,6-fused bicyclic dipeptide scaffolds (**5**) that possessed utility as ICE inhibitors. The core bicyclic scaffold was easily prepared utilizing an olefin metathesis reaction for construction of the 8-membered ring. Multiple compounds from both structural classes were identified with good enzyme (<10 nM) and whole cell potency (<150 nM). Further pharmacokinetic and in vivo evaluation of these compounds will be reported elsewhere.

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15. Data for **5a**: ^{1}H NMR ($CDCl_3$) δ 5.92 (br d, J = 6.9 Hz, 1H), 5.66 (br s, 2H), 4.90 (m, 2H), 4.55 (d, J = 11.7 Hz, 1H), 4.30 (br d, J = 11.8 Hz, 1H), 3.86–3.64 (m, 3H), 3.77 (s, 3H), 2.92 (m, 2H), 2.48 (m, 1H), 2.31 (br d, J = 16.5 Hz, 1H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) 172.9, 170.5, 155.5, 128.9, 124.3, 80.0, 70.7, 67.9, 52.6, 51.6, 51.2, 51.0, 36.3, 33.4, 28.6 (3C); MS 355 $(M+H)^+$.
16. Data for **5b**: ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 5H), 5.86 (br d, J = 6.3 Hz, 1H), 5.63 (m, 2H), 5.17 (br s, 2H), 5.06 (d, J = 4.2 Hz, 1H), 4.92 (m, 1H), 4.64 (d, J = 13.8, 1H), 4.40 (m, 1H), 3.98 (m, 1H), 3.65 (m, 1H), 3.25 (m, 2H), 2.92 (m, 1H), 2.60–2.30 (m, 3H), 1.45 (s, 9H); MS 488 $(M+H)^+$.
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18. Data for **15i**: ^{1}H NMR (CD_3OD) δ 8.50 (s, 1H), 7.98 (m, 4H), 7.62 (m, 2H), 5.80 (m, 1H), 5.69 (m, 1H), 4.75 (d, J = 2.1 Hz, 1H), 4.68 (m, 1H), 4.55 (m, 1H), 4.49 (d, J = 6.2 Hz, 1H), 4.37 (m, 1H), 3.65 (dd, J = 12, 4.5 Hz, 1H), 3.36–3.21 (m, 2H), 2.88 (m, 1H), 2.64 (m, 1H), 2.54 (m, 4H); MS 494 $(M+H)^+$.
19. Data for **16j**: ^{1}H NMR (CD_3OD) δ 8.48 (s, 1H), 8.02 (m, 1H), 7.98 (m, 3H), 7.60 (m, 2H), 5.18 (m, 2H), 4.71 (m, 1H), 4.55 (d, J = 12.8 Hz, 1H), 4.33 (m, 1H), 4.23 (d, J = 13.2 Hz, 1H), 3.69 (d, J = 12.4 Hz, 1H), 3.33 (m, 1H), 3.08 (m, 1H), 3.02 (s, 3H), 2.61 (m, 2H), 2.20 (m, 2H), 1.90 (m, 4H), 1.60 (m, 2H); MS 573 $(M+H)^+$.
20. Manuscript in preparation.
21. The isolated caspase enzyme (caspase-1, -3, and -8) assays were performed in a 96-well format using fluorogenic substrates, enzymes, and control peptide inhibitors purchased from BioMol Research Laboratories (Plymouth Meeting, PA). The assay was conducted according to the manufacturer's instructions. Enzyme inhibition was monitored over 30 min at 37 °C by measuring fluorescence using a BMG Fluostar plate reader (excitation filter 390 nm, emission filter 460 nm). IC_{50} values were calculated based on the equation $IC_{50} = [I]/(V_o/V_i) - 1$, where V_i is the initial velocity of substrate cleavage in the presence of inhibitor at concentration [I], and V_o is the initial velocity in the absence of inhibitor.
22. A suspension of human monocytic cells (THP-1, ATCC strain TIB202, 2×10^6/ml in RPMI 1640 medium from Gibco-BRL) was plated in 96-well plates, incubated with or without compounds (administered as solutions in DMSO, such that test concentrations ranged from 1 nM to 10 μM) for 15 min, and then stimulated with LPS (1 μg/ml) for a total of 4 h. Cells were centrifuged and the conditioned media were collected to quantify the release of IL-1β by an ELISA measurement according to the manufacturer's instructions (R&D Systems, catalog No. DLB50) or stored at −20 °C for future use.